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China - Peoples Republic of

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National Food Safety Standard - Amaranth

Report Categories:

FAIRS Subject Report

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Report Highlights:

On May 5, 2010, China notified the WTO of National Food Safety Standard: Food Additives – Amaranth as SPS/N/CHN/275. This measure applies to the production, circulation, supervision and management of the food additive amaranth. It specifies the scope, requirements and testing methods. The date for submission of final comments to China is May 20, 2010. The proposed date of entry is May 30, 2010. Contact information on where to send comments is inside the report. This report is an INFORMAL translation of this document.

Executive Summary:

On May 5, 2010, China notified the WTO of National Food Safety Standard: Food Additives – Amaranth as SPS/N/CHN/275. This measure applies to the production, circulation, supervision and management of the food additive amaranth. It specifies the scope, requirements and testing methods. The date for submission of final comments to China is May 20, 2010. The proposed date of entry is May 30, 2010. This report is an INFORMAL translation of this document.

Comments can be sent to the China WTO SPS Enquiry Point at: SPS@aqsic.gov.cn.

This report contains an UNOFFICIAL translation of National Standard on Determination of Amaranth in Foods.

General Information:

BEGIN TRANSLATION

National Food Safety Standard

GB 4479.1-XXX

Food Additive - Amaranth

National Food Safety Standard

(Draft for Comment)

Issued on xx-xx-xxxx

Implemented on xx-xx-xxxx

Issued by the Ministry of Health
of the People's Republic of China

Foreword

This Standard is modified in relation to "Food amaranth No. 2" in Japan's Specifications and Standards for Food Additives (Edition 8).

Main technical differences between this Standard and "Food Amaranth No. 2" in the eighth edition of Japan's Specifications and Standards for Food Additives are specified in Annex E.

This Standard supersedes GB 4479.1-1999 Food Additive – Amaranth.

Compared with GB 4479.1-1999, this Standard has the following main changes:

- adding CI No., INS No. and CAS No.;
- canceling requirement of 60.0 % in the original standard;
- modifying appearance from "red brown to dark red brown powder" to "red brown to dark

red brown powder or granule”;

- modifying content of water insoluble matters from $\leq 0.30\%$ to $\leq 0.20\%$;
- modifying method of identification test;
- modifying permissible difference for parallel determinations by spectrophotometric colorimetric method from 2.0% to 1.0% ;
- adding control requirements and test methods for unreacted intermediates;
- adding control requirements and test methods for unsulfonated aromatic primary amine (based on aniline);
- modifying chemical half-limit method for arsenic into atomic absorption method; and
- modifying content requirement for heavy metal (based on lead) into control requirement for lead and modifying test method into atomic absorption method.

Annexes A, B and C of this Standard are normative, and Annex E of this Standard is informative.

This Standard supersedes the following previous editions:

- GB 4479.1-1986, GB 4479.1-1996 and GB 4479.1-1999

National Food Safety Standard

Food Additive Amaranth

1 Scope

This Standard is applicable to quality control of amaranth products made by coupling diazotized sodium 4-amino-1-naphthalenesulfonate with 2-naphthol-3,6-disulfonic acid sodium salt.

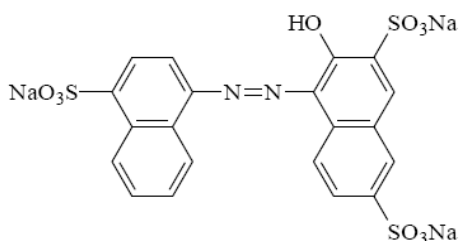
2 Normative references

Documents referenced in this Standard are indispensable for the application of this Standard. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

3 Chemical name, structural formula, molecular formula, relative molecular mass, INS No. and CAS No.

Chemical name: trisodium-2-hydroxy-1-(4-sulphonato-1-naphthylazo) naphthelene- 3-6-disulfonate

Structural formula:



Molecular formula: C₂₀H₁₁N₂Na₃O₁₀S₃

Relative molecular mass: 604.48 (based on 2007 International Relative Atomic Mass)

CI No.: C.I.16185

INS No.: 123

CAS No.: 915-67-3

4 Technical requirements

Technical requirements of amaranth shall be in accordance with Table 1.

Table 1 Technical requirements of amaranth

Items	Requirement	Test method
Appearance	Red brown to dark red brown powder or particle	Visual inspection under natural light
Amaranth, w/%	≥ 85.0	A.3 in Annex A
Loss on drying, chloride and sulfate (based on sodium salt), w/%	≤ 15.0	A.4 in Annex A
Water insoluble matter, w/%	≤ 0.20	A.5 in Annex A
subsidiary colors, w /%	≤ 3.0	A.6 in Annex A
Total unreacted intermediates, w /%	≤ 0.50	A.7 in Annex A
Unulfonated aromatic primary amine (based on aniline), w/%	≤ 0.01	A.8 in Annex A
Arsenic /(mg/kg)	≤ 1.0	A.9 in Annex A
Lead /(mg/kg)	≤ 10.0	A.10 in Annex A

Annex A

(Normative)

Test Method

A.1 General requirements

Reagents and water used in this Standard, unless otherwise stated, are analytically pure reagents and grade III water specified in GB 6682-2008. Standard solution, impurity standard solution, preparations and products required in the tests of this Standard, unless otherwise stated, shall be prepared and calibrated according to requirements of GB/T 601, GB/T 602 and GB/T 603. Test results shall be judged in accordance with 4.3.3 Round-off comparison method in GB/T 8170-2008.

A.2 Identification

A.2.1 Reagents and solutions

- Sulfuric acid;
- Ammonium acetate: 1.5 g/L.

A.2.2 Apparatus

- Spectrophotometer;
- Cuvette: 10 mm.

A.2.3 Identification method

Weigh about 0.1 g of the sample (accurate to 0.01 g), dissolve in 100 mL of water, the resulting

solution is red clear solution .

Weigh about 0.2 g of the sample (accurate to 0.01 g), add 20 mL of sulfuric acid, the solution develops purple, add 2 or 3 drops of the solution to 5 mL of water, the water develops red.

Weigh about 0.1 g of the sample (accurate to 0.01 g), dissolve in 100 mL of ammonium acetate, add ammonium acetate solution to 1 mL of the resulting solution to make 100 mL, and the maximum absorption wavelength of the solution is $520 \text{ nm} \pm 2 \text{ nm}$.

A.3 Determination of amaranth

A.3.1 Titanium trichloride titration method (arbitrary method)

A.3.1.1 Method summary

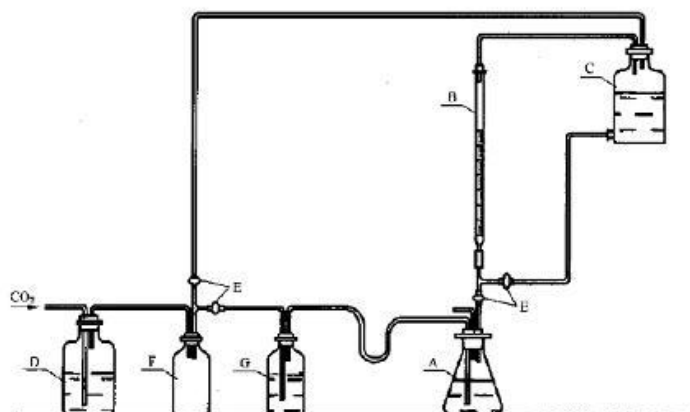
In acid medium, azo group in amaranth is reduced and decomposed by titanium trichloride and content of amaranth is calculated according to consumption of titanium trichloride standard titration solution.

A.3.1.2 Reagents and materials

- a) Trisodium citrate;
- b) Titanium trichloride standard titration solution: $c(\text{TiCl}_3) = 0.1 \text{ mol/L}$ (freshly prepared, see Annex B for preparation method);
- c) Carbon dioxide in cylinder.

A.3.1.3 Apparatus

See Fig. A.1.



A – Conical flask (500 mL);

B – Brown buret (50 mL);

C – Black paper wrapped glass bottle with bottom mouth (2000 mL);

D – Container with mixed solution of equal volumes of 100 g/L ammonium carbonate solution and 100 g/L ferrous sulfate solution (5000 mL);

E – Piston;

F – Empty bottle;

G – Gas washing bottle filled with water.

Fig. A.1 Apparatus for titanium trichloride titration method

A.3.1.4 Determination procedures

Weigh about 0.5 g of the sample (accurate to 0.0001 g), place in a 500 mL conical flask, dissolve in 50 mL of water which has been freshly boiled and cooled to room temperature, add 15 g of trisodium citrate and 150 mL of freshly boiled water, shake vigorously to dissolve, install apparatus according to Fig. 1, introduce carbon dioxide under liquid level while heating to boiling, titrate with titanium trichloride standard titration solution till the inherent color disappears.

A.3.1.5 Result calculation

Amaranth content is calculated according to formula (A.1) based on mass fraction w_1 and its value is expressed in %:

$$w_1 = \frac{c(V/1000)(M/4)}{m_1} \times 100 \dots \dots \dots (A.1)$$

where:

c – accurate value of concentration of titanium trichloride standard titration solution, expressed in mol/L;

V – accurate value of volume of titanium trichloride standard titration solution consumed for titrating the sample, expressed in mL;

M – value of molar mass of amaranth, expressed in g/mol [$M(C_{20}H_{11}N_2Na_3O_{10}S_3) = 604.48$];

m_1 – value of mass of sample, expressed in g.

Calculation result is rounded to 0.1.

A.3.1.6 Permissible difference

Absolute difference between two parallel determination results is not more than 1.0 % (mass fraction) and the arithmetic mean is taken as determination result.

A.3.2 Spectrophotometric colorimetric method

A.3.2.1 Method summary

Dissolve the sample and amaranth standard substance with known content in water respectively, measure absorbance respectively at the maximum absorption wavelength, and then calculate the content.

A.3.2.2 Reagents and solutions

- a) Ammonium acetate solution: 1.5 g/L;
- b) amaranth standard substance: $\geq 85.0\%$ (mass fraction, determined according to A.3.1 in this Standard).

A.3.2.3 Apparatus

- a) Spectrophotometer;
- b) Cuvette: 10 mm.

A.3.2.4 Preparation of amaranth standard sample solution

Weigh about 0.5 g of amaranth standard substance (accurate to 0.0001 g), dissolve in a proper amount of water, transfer to a 1000 mL volumetric flask, add ammonium acetate solution and dilute to volume, shake up. Pipette 10 mL to a 5000 mL volumetric flask, add ammonium acetate solution and dilute to volume, shake up for use.

A.3.2.5 Preparation of amaranth sample solution

The weighing and operation methods are the same as those for preparation of standard sample solution.

A.3.2.6 Determination procedures

Place amaranth standard sample solution and amaranth sample solution in 10 mm cuvettes respectively, measure absorbance respectively at the maximum absorption wavelength, and use ammonium acetate solution as reference solution.

A.3.2.7 Result calculation

Amaranth content is calculated according to formula (A.2) based on mass fraction w_1 and its value is expressed in %:

$$w_1 = \frac{Am_0}{A_0m} \times w_0 \dots\dots\dots (A.2)$$

where:

A – value of absorbance of amaranth sample solution;

m_0 – value of mass of amaranth standard substance, expressed in g;

A_0 – value of absorbance of amaranth standard sample solution;

M – value of mass of sample, expressed in g;

w_0 – value of amaranth standard substance, expressed in % (mass fraction).

Calculation result is rounded to 0.1.

A.3.2.8 Permissible difference

Absolute difference between two parallel determination results is not more than 1.0 % (mass fraction) and the arithmetic mean is taken as determination result.

A.4 Determination of total of loss on drying, chloride (based on NaCl) and sulfate (based on Na_2SO_4)

A.4.1 Determination of loss on drying

A.4.1.1 Determination procedures

Weigh about 2 g of the sample (accurate to 0.001 g), place in a weighing bottle with constant weight, bake in a

135 °C constant temperature oven to constant weight.

A.4.1.2 Result calculation

Loss on drying is calculated according to formula (A.3) based on mass fraction w_2 and its value is expressed in %:

$$w_2 = \frac{m_2 - m_3}{m_2} \times 100 \dots\dots\dots (A.3)$$

where:

m_2 – value of sample before drying, expressed in g;

m_3 – value of mass of sample after drying to constant weight, expressed in g.

Calculation result is rounded to 0.1.

A.4.1.3 Permissible difference

Absolute difference between two parallel determination results is not more than 0.2 % (mass fraction) and the arithmetic mean is taken as determination result.

A.4.2 Determination of chloride (based on NaCl)

A.4.2.1 Reagents and solutions

- a) Nitrobenzene;
- b) Activated carbon: type 767 injection powder;
- c) Nitric acid solution: 1+1;
- d) Silver nitrate solution: $c(\text{AgNO}_3) = 0.1 \text{ mol/L}$;
- e) Ammonium ferric sulfate solution:

Preparation method: weigh about 14 g of ammonium ferric sulfate, dissolve in 100 mL of water, filter, add 10 mL of nitric acid and store in a brown bottle;

- f) Ammonium thiocyanate standard titration solution: $[c(\text{NH}_4\text{CNS}) = 0.1 \text{ mol/L}]$.

A.4.2.2 Preparation of sample solution

Weigh about 2 g of the sample (accurate to 0.001 g), dissolve in 150 mL of water, add about 15 g of activated carbon, boil mildly for 2 to 3 min, add 1 mL of nitric acid solution, shake up constantly and stand for 30 min (while shaking during this period). Filter with dry filter paper. Add 5 g of activated carbon if the filtrate is colored, stand for 1 h while shaking constantly, filter with dry filter paper (if filtrate is still colored, replace activated carbon, operate repeatedly till the filtrate is colorless). Wash activated carbon with 10 mL of water each time, merge filtrate and pipette to a 200 mL volumetric flask, dilute to volume with water and shake up. The solution is used for determination of contents of chloride and sulfate.

A.4.2.3 Determination procedures

Pipette 50 mL of sample solution to a 500 mL conical flask, add 2 mL of nitric acid solution and 10 mL of silver nitrate solution (add more when chloride content is larger) and 5 mL of nitrobenzene, shake vigorously till silver chloride condenses, add 1 mL of ammonium ferric sulfate, titrate excessive silver nitrate to end point with ammonium thiocyanate standard titration solution and stand for 1 min. Meantime, perform a blank test by the same method.

A.4.2.4 Result calculation

Chloride content (based on NaCl) is calculated according to formula (A.4) based on mass fraction w_3 and its value is expressed in %:

$$w_3 = \frac{c_1[(V_1 - V_0)/1000]M_1}{m_4(50/200)} \times 100 \dots \dots \dots (A.4)$$

where:

c_1 – accurate value of concentration of ammonium thiocyanate standard titration solution, expressed in mol/L;

V_1 – accurate value of volume of ammonium thiocyanate standard titration solution consumed for titrating blank solution, expressed in mL;

V_0 – accurate value of volume of ammonium thiocyanate standard titration solution consumed for

titrating sample solution, expressed in mL;

M1 – value of molar mass of sodium chloride, expressed in g/mol [$M(\text{NaCl}) = 58.4$];

m4 – value of mass of sample, expressed in g.

Calculation result is rounded to 0.1.

A.4.2.5 Permissible difference

Absolute difference between two parallel determination results is not more than 0.3 % (mass fraction) and the arithmetic mean is taken as determination result.

A.4.3 Determination of sulfate (based on Na_2SO_4)

A.4.3.1 Reagents and solutions

a) Sodium hydroxide solution: 0.2 g/L;

b) Hydrochloric acid solution: 1 + 1999;

c) Barium chloride standard titration solution: $c(1/2\text{BaCl}_2) = 0.1 \text{ mol/L}$ (see Annex C for preparation method);

d) Phenolphthalein indicator solution: 10 g/L;

e) Rhodizonic acid disodium salt indicator solution: weigh 0.1 g of rhodizonic acid, dissolve in 10 mL of water (prepare freshly).

A.4.3.2 Determination procedures

Pipette 25 mL of sample solution (A.4.2.2 of this Standard) to a 250 mL conical flask, add one drop of phenolphthalein indicator solution, add sodium hydroxide solution dropwise till color of the solution shall not develop pink, shake up, titrate with barium chloride standard titration solution while shaking constantly after dissolving, use rhodizonic acid disodium salt indicator solution as external indicator solution, and the end point is that rhodizonic spots present for 2 min at the intersection between reaction solution and indicator solution on the filter paper.

Meantime, perform a blank test by the same method.

A.4.3.3 Result calculation

Sulfate content (based on Na_2SO_4) is calculated according to formula (A.5) based on mass fraction w_4 and its value is expressed in %:

$$w_4 = \frac{c_2[(V_2 - V_3)/1000](M_2/2)}{m_4(25/200)} \times 100 \dots \dots \dots (A.5)$$

where:

c_2 – accurate value of concentration of barium chloride standard titration solution, expressed in mol/L;

V_2 – accurate value of volume of barium chloride standard titration solution consumed for titrating sample solution, expressed in mL;

V_3 – accurate value of volume of barium chloride standard titration solution consumed for titrating blank solution, expressed in mL;

M_2 – value of molar mass of sodium sulfate, expressed in g/mol [$M(\text{Na}_2\text{SO}_4) = 142.04$];

m_4 – value of mass of sample, expressed in g.

Calculation result is rounded to 0.1.

A.4.3.4 Permissible difference

Absolute difference between two parallel determination results is not more than 0.2 % (mass fraction) and the arithmetic mean is taken as determination result.

A.4.4 Result calculation of total of loss on drying, chloride (based on NaCl) and sulfate (based on Na₂SO₄)

Total of loss on drying, chloride (based on NaCl) and sulfate (based on Na₂SO₄) is calculated according to formula (A.6) based on mass fraction w_5 and its value is expressed in %:

$$w_5 = w_2 + w_3 + w_4 \dots\dots\dots (A.6)$$

where:

- content of loss on drying, expressed in % (mass fraction);
- content of chloride (based on NaCl), expressed in % (mass fraction);
- content of sulfate (based on Na₂SO₄), expressed in % (mass fraction).

Calculation result is rounded to 0.1.

A.5 Determination of water insoluble matter

A.5.1 Apparatus

- a) Sintered glass crucible: G4, aperture: 5 µm to 15 µm;
- b) Constant temperature oven.

A.5.2 Determination procedures

Weigh about 3 g of the sample (accurate to 0.001 g), place in a 500 mL beaker, add 250 mL of hot water at 50 °C - 60 °C, dissolve and filter through sintered glass crucible which has been baked to constant at 135 °C, fully wash with hot water to colorless, and dry to constant weight in 135 °C constant temperature oven.

A.5.3 Result calculation

Water insoluble matter is calculated according to formula (A.7) based on mass fraction w_6 and its value is expressed in %:

$$w_6 = \frac{m_6}{m_5} \times 100 \dots\dots\dots (A.7)$$

where:

- m_6 – value of mass of dried water insoluble matter, expressed in g;
- m_5 – value of mass of sample, expressed in g.

Calculation result is rounded to 0.01.

A.5.4 Permissible difference

Absolute difference between two parallel determination results is not more than 0.05 % (mass fraction) and the arithmetic mean is taken as determination result.

A.6 Determination of subsidiary colors

A.6.1 Method summary

Separate all components by paper chromatography, elute and quantify by spectrophotometry.

A.6.2 Reagents and solutions

- a) Anhydrous alcohol;
- b) N-butyl alcohol;
- c) Acetone solution: 1+1;
- d) Ammonia water: 4+96;
- e) Sodium bicarbonate solution: 4 g/L.

A.6.3 Apparatus and instruments

- a) Spectrophotometer;
- b) Chromatography filter paper: No.1 medium speed, 150 mm×250 mm;
- c) Chromatography tank: $\phi 240$ mm × 300 mm;
- d) Micro sample injector: 100 μ L;
- e) Nessler tube: 50 mL, with ground glass stopper;
- f) Sintered glass funnel: G3, aperture: 15 μ m to 40 μ m;
- g) 50 mm cuvette;
- h) 10 mm cuvette.

A.6.4 Determination procedures

A.6.4.1 Conditions for paper chromatography

- a) Developing solvent: n-butyl alcohol + anhydrous alcohol + ammonia water solution = 6+2+3;
- b) Temperature: 20 °C - 25 °C.

A.6.4.2 Preparation of sample solution

Weigh 1 g of the sample (accurate to 0.001 g), place in a beaker, add a proper amount of water and dissolve, transfer to a 100 mL volumetric flask, dilute to volume, shake up for use. The concentration of this sample solution is 1 %.

A.6.4.3 Preparation of sample eluate

Absorb 100 μ L of sample solution by micro sample injector, inject evenly on the baseline 25 mm away from baseline of filter paper and form a straight line with width not more than 5 mm and length of 130 mm, blow dry by a blower. Develop filter paper in a chromatography tank with pre-prepared developing solvent, immerge bottom edge of filter paper 10 mm under the level of the developing solvent, and the end point is that the front line of developing solvent rises to 150 mm or subsidiary colors are separated to satisfaction. Take out chromatography filter paper, blow dry with cold air.

Develop blank filter paper under same conditions, and this blank filter paper must be cut from adjacent part on the same filter paper as the filter paper developed by the foregoing procedures. Diagram of paper chromatography of subsidiary colors is shown in Fig. A.2.

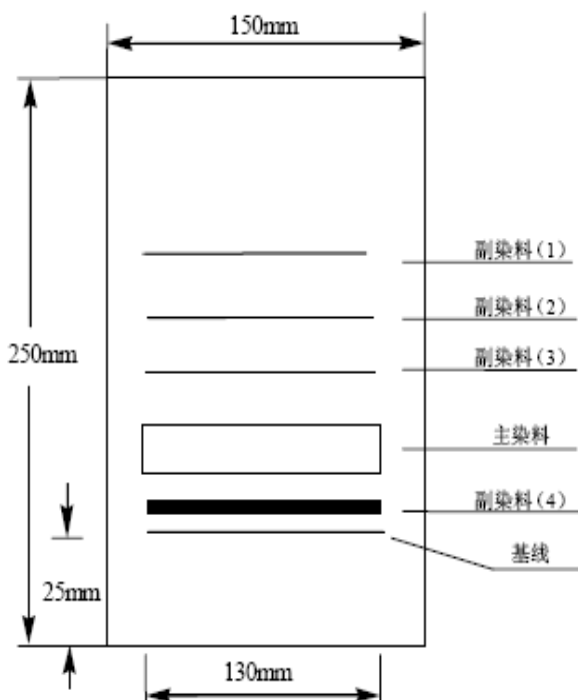


Fig. A.2 Diagram of paper chromatography of subsidiary colors

Cut various developed subsidiary colors and parts of filter paper corresponding to the subsidiary colors on the blank filter paper at the same size, cut into 5 mm×15 mm strips, place into 50 mL Nessler cuvettes separately, add accurately 5 mL of acetone solution, shake for 3 to 5 min, add accurately 20 mL of sodium bicarbonate solution, shake vigorously and naturally filter in G3 sintered glass funnel separately. The filtrate must be clear and free of any suspension. Obtain eluates of all subsidiary colors and blank solution separately. Measure absorbance values of eluates of subsidiary colors on the spectrophotometer at the maximum absorption wavelength of subsidiary colors by 50 mm cuvette.

When measuring absorbance on the spectrophotometer, mixed solution of 5 mL of acetone solution and 20 mL of sodium bicarbonate solution is used as reference solution.

A.6.4.4 Preparation of standard solution

Absorb 6 mL of 1 % sample solution, transfer to a 100 mL volumetric flask, dilute to volume and shake up. This solution is standard solution.

A.6.4.5 Preparation of standard eluate

Absorb 100 μ L of standard solution by micro sample injector, inject evenly on the baseline 25 mm away from bottom edge of filter paper and blow dry by a blower. Develop filter paper in a chromatography tank with pre-prepared developing solvent, take out the filter paper when the front line of developing solvent rises 40 mm, blow dry with cold air, cut all developed parts of colors, extract according to method specified in A.6.4.3 of this Standard and obtain standard eluate. Measure absorbance value at the maximum absorption wavelength by 10 mm cuvette. Meantime, develop blank filter paper under same conditions, operate by the same method and then measure absorbance value of eluate.

A.6.4.6 Result calculation

Content of subsidiary colors is calculated according to formula (A.8) based on mass fraction w_7 and its value is expressed in %:

$$w_7 = \frac{A_1 - b_1 + \dots + A_n - b_n}{(A_s - b_s)(100/6)} \times 5 \times S \dots \dots \dots (A.8)$$

where:

$A_1 \dots, A_n$ -- absorbance values of eluates of all subsidiary colors measured at 50 mm beam path distance;

$b_1 \dots, b_n$ -- absorbance values of control blank eluates of all subsidiary colors measured at 50 mm beam path distance;

A_s -- absorbance value of standard eluate measured at 10 mm beam path distance;

b_s -- absorbance value of standard control blank eluate measured at 10 mm beam path distance;

5 -- ratio of being converted into 10 mm beam path distance;

100/6 -- ratio of standard eluate converted into 1 % sample solution;

S -- content of sample, expressed in % (mass fraction).

Calculation result is rounded to 0.1.

A.6.4.7 Permissible difference

Absolute difference between two parallel determination results is not more than 0,2 % (mass fraction) and the arithmetic mean is taken as determination result.

A.7 Determination of total unreacted intermediates

A.7.1 Method summary

Use reverse liquid chromatography, measure unreacted intermediates by external standard method respectively, and finally calculate mass fraction of total unreacted intermediates.

A.7.2 Reagents and solutions

- Methanol;
- ammonium acetate solution: 2 g/L;
- sodium-1-naphthylamine-4-sulfonate;
- sodium 7-hydroxy-1,3-naphthalenedisulfonate;
- sodium 3-hydroxy-2,7-naphthalenedisulfonate;
- sodium 6-hydroxy-2-naphthalenesulfonate;
- sodium 6-hydroxy-1,3,6-naphthalenetrisulfonate.

A.7.3 Apparatus and instruments

- Liquid phase chromatograph: infusion pump – the flow range is 0.1 mL/min to 5.0 mL/min, and flow stability within this range is ± 1 %.

Detector – multi-wavelength UV spectrophotometric detector or UV spectrophotometric detector with equal function;

- Chromatographic column: 150 mm long stainless steel column with the inside diameter of 4.6 mm, stationary phase of C18 and particle diameter of 5 μm ;

- Chromatographic work station or integrator;

j) Ultrasonic generator;

k) Dosing ring: 20 µL.

A.7.4 Chromatographic conditions

a) Detection wavelength: 238 nm;

b) Column temperature: 30 °C;

c) Mobile phase: A. ammonium acetate solution; B. methanol;

Concentration gradient: A:B (100:0) for 5min, and then concentration gradient declines from A:B (100:0) to A:B (70:30) for 50 min;

d) Flow rate: 1.0 mL/min;

e) Sample size: 20 µL.

Select optimum analysis condition according to instrument, shake up mobile phase and degas by ultrasonic generator.

A.7.5 Preparation of sample solution

Weigh about 0.1 g of amaranth sample (accurate to 0.0001 g), add ammonium acetate solution for dissolving to make a constant volume of 100 mL.

A.7.6 Preparation of standard solution

Respectively weigh about 0.01 g (accurate to 0.0001 g) of sodium-1-naphthylamine-4-sulfonate, sodium 7-hydroxy-1, 3-naphthalenedisulfonate, sodium 3-hydroxy-2, 7-naphthalenedisulfonate, sodium 6-hydroxy-2-naphthalenesulfonate and sodium 6-hydroxy-1,3,6-naphthalenetrisulfonate which have been dried in vacuum dryer for 24 h. Respectively dissolve with ammonium acetate solution to make 100 mL. Then respectively absorb 10.0 mL, 5.0 mL, 2.0 mL and 1.0 mL of aforesaid solution, add ammonium acetate solution to make 100 mL and prepare into series standard solutions.

A.7.7 Determination procedures

Under chromatographic conditions specified in A.7.4 of this Standard, respectively absorb sample solution and series standard solutions by micro sample injector, fully inject into dosing ring for chromatographic detection, and calculate results after discharge of the last component. Measure peak areas of all standard solution substances and draw standard curves respectively. Measure peak areas of sodium-1-naphthylamine-4-sulfonate, sodium 7-hydroxy-1,3-naphthalenedisulfonate, sodium 3-hydroxy-2,7-naphthalenedisulfonate, sodium 6-hydroxy-2-naphthalenesulfonate and sodium 6-hydroxy-1,3,6-naphthalenetrisulfonate in sample solution and calculate mass fractions of respective unreacted intermediates according to the standard curves. (See Annex D for chromatogram).

A.7.8 Result calculation

Total of unreacted intermediates is calculated according to formula (A.9) based on mass fraction w_{13} and its value is expressed in %:

$$w_{13} = w_8 + w_9 + w_{10} + w_{11} + w_{12} \dots \dots \dots (A.9)$$

where:

- content of sodium-1-naphthylamine-4-sulfonate, expressed in % (mass fraction);

- content of sodium 7-hydroxy-1,3-naphthalenedisulfonate, expressed in % (mass fraction);
- content of sodium 3-hydroxy-2,7-naphthalenedisulfonate, expressed in % (mass fraction);
- content of sodium 6-hydroxy-2-naphthalenesulfonate, expressed in % (mass fraction);
- content of sodium 6-hydroxy-1,3,6-naphthalenetrisulfonate, expressed in % (mass fraction);

A.8 Determination of unsulfonated aromatic primary amine (based on aniline)

A.8.1 Method summary

Compare sample and aniline standard solution by spectrophotometry after diazo coupling.

A.8.2 Reagents and solutions

- Ethyl acetate;
- Hydrochloric acid solution: 1+10;
- Hydrochloric acid solution: 1+3;
- Potassium bromide solution: 500 g/L;
- Sodium carbonate solution: 200 g/L;
- Sodium hydroxide solution: 40 g / L;
- Sodium hydroxide solution: 4 g/L;
- R salt solution: 20 g/L;
- sodium nitrite solution: 3.52 g/L;
- Aniline standard solution: 0.1000 g/L;

Preparation: weigh 0.5000 g of freshly distilled aniline by a small beaker, transfer to a 500 mL volumetric flask, wash beaker for three times with 150mL of (1+3) hydrochloric acid solution, merge into the 500 mL volumetric flask and dilute to volume with water. Pipette 25 mL of such solution to a 250 mL volumetric flask and dilute to volume with water. Concentration of aniline in this solution is 0.1000 g/L.

A.8.3 Apparatus and instruments

- Visible spectrophotometer;
- cuvette: 40 mm.

A.8.4 Preparation of sample extract solution

Weigh about 2.0 g of the sample (accurate to 0.001 g) and place in a 150 mL beaker, add 100 mL of water and 5 mL of (40 g/L) sodium hydroxide solution, stir in lukewarm bath to fully dissolving. Transfer such solution to separating funnel and wash the beaker with a proper amount of water. Extract twice with each time consuming 50 mL of ethyl acetate and then merge extract solution. Wash extract solution with 10 mL of (4 g/L) sodium hydroxide solution to remove trace colors. Reversely extract ethyl acetate solution for three times with each time consuming 10 mL of (1+3) hydrochloric acid solution. Merge such hydrochloric acid extract solution, dilute to 100 mL with water and shake up. This solution is sample extract solution.

A.8.5 Preparation of standard control solution

Pipette 2.0 mL of aniline standard solution to a 100 mL volumetric flask, dilute to volume with (1+10) hydrochloric acid solution and fully mix. This solution is standard control solution.

A.8.6 Preparation of diazo coupling solution

Respectively absorb 10 mL of sample extract solution and 10 mL of standard control solution, respectively transfer to transparent and clean test tubes, immerse into beakers with ice-water mixture and cool for 10 min. Respectively add 1 mL of potassium bromide solution and 0.5 mL of sodium nitrite solution to test tubes, shake up slightly, stand in ice-water bath and cool for 10 min, and perform diazo reaction. Respectively transfer 1 mL of R salt solution and 10 mL of sodium carbonate solution to 25 mL volumetric flasks. Add aniline diazonium salt solution in aforesaid test tubes to volumetric flasks with R salt solution, shake volumetric flasks vigorously while adding, wash test tubes clean with a proper amount of water, merge into volumetric flask and dilute to volume with water. Fully mix and then stand in dark place for 15 min. Such solutions are respectively sample diazo coupling solution and standard diazo coupling solution.

A.8.7 Preparation of reference solution

Pipette 10 mL of (1+10) hydrochloric acid solution, 10 mL of sodium carbonate solution and 1 mL of R salt solution to a 25 mL volumetric flask and dilute to volume with water. Such solution is reference solution.

A.8.8 Determination procedures

Place sample diazo coupling solution and standard diazo coupling solution into cuvettes respectively and measure absorbance A_a and A_b by spectrophotometer at the absorption wavelengths of 510 nm with solution prepared in A.8.7 of this Standard as reference solution.

A.8.9 Result determination

It is acceptable if A_b is not more than A_a .

A.9 Determination of arsenic

A.9.1 Method summary

Prepare amaranth into sample solution after digestion with wet method and then measure content of arsenic with atomic absorption spectrometry.

A.9.2 Reagents and solutions

- a) Nitric acid;
- b) Sulfuric acid solution: 1+1;
- c) nitric acid-perchloric acid mixed solution: 3+1;
- d) Arsenic (As) standard solution: prepare and calibrate in accordance with GB/T 602, then dilute and prepare into three standard solutions with different arsenic concentrations according to requirements of apparatus used;
- e) Sodium hydroxide solution: 1 g/L;
- f) Sodium borohydride solution: 8g/L (solvent is 1g/L sodium hydroxide solution);
- g) Hydrochloric acid: 1+10;
- h) Potassium iodide solution: 200 g/L.

A.9.3 Apparatus

Atomic absorption spectrometer

Reference conditions of apparatus: analysis line wavelength of arsenic hollow cathode lamp: 193.7 nm; slit: 0.5 nm – 1.0 nm; lamp current: 6 mA – 10 mA;

Flow rate of carrier gas: argon gas, 250 mL/min;

Temperature of atomizer: 900 °C.

A.9.4 Determination procedures

A.9.4.1 Sample digestion

Weigh about 1.0 g of the sample (accurate to 0.001 g), place in a 250 mL Erlenmeyer flask or round bottomed flask, add 10 mL - 15 mL of nitric acid and 2 mL of sulfuric acid solution, shake up and evict nitrogen dioxide gas by heating with small fire, stop heating when solution develops brown, cool down and then add 5 mL of nitric acid-perchloric acid mixed solution, heat with strong fire till the solution becomes transparent and colorless or develops slight yellow. If the solution is still nontransparent, cool down and then add another 5 mL of nitric acid-perchloric acid mixed solution, keep heating till the solution becomes transparent and colorless or develops slight yellow with white smoke (avoid carbonization due to burning out), stop heating, cool down, add 5 mL of water and heat to boiling, remove residual nitric acid-perchloric acid (add water and heat to boiling again if necessary), keep heating till white smoke develops, stand for 10 min, transfer to a 100 mL volumetric flask after cooling (filter when the solution is turbid or precipitate or mechanic impurities appear) and dilute to volume with hydrochloric acid solution.

Meantime, prepare blank solution by the same method.

A.9.4.2 Determination

Transfer 25 mL of digested sample solution to a 50 mL volumetric flask, add 5 mL of potassium iodide solution, dilute to volume with hydrochloric acid solution, shake up and stand for 15 min.

Meantime, prepare blank test solution with blank solution by the same method.

Turn on apparatus, after instrument and arsenic hollow cathode lamp are fully preheated and baseline is stable, use sodium borohydride solution as hydride reducing agent, respectively inject standard blank, standard solution, sample blank test solution and sample solution in sequence according to computer instruction. After completion of test, computer automatically generates working curve and arsenic concentration of sample solution with sample blank deducted, input sample information (name, weight, dilution volume, etc.), then content of arsenic in sample is automatically calculated.

A.9.4.3 Permissible difference

Absolute difference between two parallel determination results is not more than 0,1 (mg/kg) and the arithmetic mean is taken as determination result.

A.10 Determination of lead

A.10.1 Method summary

Prepare amaranth into sample solution after digestion with wet method and then measure content of lead with atomic absorption spectrometry.

A.10.2 Reagents and solutions

a) Lead (Pb) standard solution: prepare and calibrate in accordance with GB/T 602, then dilute and prepare into three standard solutions with different lead concentrations according to requirements of apparatus used;

b) Sodium hydroxide solution: 1 g/L;

- c) Sodium borohydride solution: 8 g/L (solvent is 1 g/L sodium hydroxide solution);
- d) Hydrochloric acid solution: 1+10.

A.10.3 Apparatus

Atomic absorption spectrometer

Reference conditions of apparatus: Method 3 in GB 5009.12 -- Flame Atomic Absorption Spectrometry.

A.10.4 Determination procedures

Sample solution and blank solution prepared in A.9.4.1 of this Standard can be directly used. Operate in accordance with Method 3 in GB 5009.12 -- Flame Atomic Absorption Spectrometry.

A.10.5 Permissible difference

Absolute difference between two parallel determination results is not more than 1.0 (mg/kg) and the arithmetic mean is taken as determination result.

Annex B

(Normative)

Preparation Method of Titanium Trichloride Standard Titration Solution

B.1 Reagents and solutions

- a) Hydrochloric acid;
- b) Ammonium ferrous sulfate;
- c) Ammonium thiocyanate solution: 200 g/L;
- d) Sulfuric acid solution: 1+1;
- e) Titanium trichloride solution;
- f) Potassium dichromate standard titration solution: $[c(1/6K_2Cr_2O_7) = 0.1 \text{ mol/L}]$, prepare and calibrate in accordance with GB /T 602.

B.2 Apparatus

See Fig. A.1 in Annex A.

B.3 Preparation of titanium trichloride standard titration solution

B.3.1 Preparation

Place 100 mL of titanium trichloride solution and 75 mL of hydrochloric acid in a 1000 mL brown volumetric flask, dilute to volume with water freshly boiled and cooled to room temperature, shake up, immediately pour into a bottle with bottom mouth away from light and store under protection of carbon dioxide gas.

B.3.2 Calibration

Weigh about 3 g of ammonium ferrous sulfate (accurate to 0.0001 g), place in a 500 mL conical flask, add 50 mL of water freshly boiled and cooled down under protection of carbon dioxide gas for dissolving and then add 25 mL of sulfuric acid solution, keep introducing carbon dioxide gas under liquid level for protection, promptly and accurately add 35 mL of potassium dichromate standard titration solution, then titrate with titanium trichloride standard solution to be calibrated to approach to the end point of calculation quantity, immediately add 25 mL of ammonium thiocyanate solution and keep titrating with titanium trichloride standard solution to be calibrated

till red color turns green (the end point). The whole titration procedure shall be under protection of carbon dioxide gas, and meantime perform a blank test.

B.3.3 Result calculation

Concentration of titanium trichloride standard solution is calculated according to formula (B.1) based on $c(\text{TiCl}_3)$ and its unit is mol/L:

$$c(\text{TiCl}_3) = \frac{cV_1}{V_2 - V_3} \dots\dots\dots (\text{B.1})$$

where:

c - accurate value of concentration of potassium dichromate standard titration solution, expressed in mol/L;

V_1 - accurate value of volume of potassium dichromate standard titration solution, expressed in mL;

V_2 - accurate value of volume of titanium trichloride standard titration solution consumed for titrating high titanium oxidized by potassium dichromate standard titration solution, expressed in mL;

V_3 - accurate value of volume of titanium trichloride standard titration solution consumed for titrating blank solution, expressed in mL.

Calculation result is rounded to 0.0001.

Above calibration shall be promptly done while performing sample analysis.

Annex C

(Normative)

Preparation Method of Barium Chloride Standard Solution

C.1 Reagents and solutions

- a) Barium chloride;
- b) Ammonia water;
- c) Sulfuric acid standard titration solution: [$c(1/2\text{H}_2\text{SO}_4) = 0.1 \text{ mol/L}$], prepare and calibrate in accordance with GB/T 601;
- d) Rhodizonic acid disodium salt indicator solution (weigh 0.1 g of rhodizonic acid, disodium salt dissolve in 10 mL of water, prepare freshly);
- e) Universal pH paper.

C.2 Preparation

Weigh 12.25 g of barium chloride, dissolve in 500 mL of water, transfer to a 1000 mL volumetric flask, dilute to volume and shake up.

C.3 Calibration method

Pipette 20 mL of sulfuric acid standard titration solution to a 250 mL volumetric flask, add 50 mL of water, neutralize with ammonia water till universal pH paper develops 8, then titrate with barium chloride standard titration solution, use rhodizonic acid disodium salt indicator solution as external indicator solution, and the end point is that rhodizonic spots present for 2 min at the intersection between reaction solution and indicator solution on the filter paper.

C.4 Result calculation

Concentration of barium chloride standard titration solution is calculated according to formula (C.1) based on $c(1/2BaCl_2)$ and its unit is mol/L:

$$c(\frac{1}{2}BaCl_2) = \frac{c_1 V_4}{V_5} \dots\dots\dots (C.1)$$

where:

c_1 - accurate value of concentration of sulfuric acid standard titration solution, expressed in mol/L;

V_4 - accurate value of volume of sulfuric acid standard titration solution, expressed in mL;

V_5 - accurate value of volume of barium chloride standard titration solution consumed, expressed in mL.

Calculation result is rounded to 0.0001.

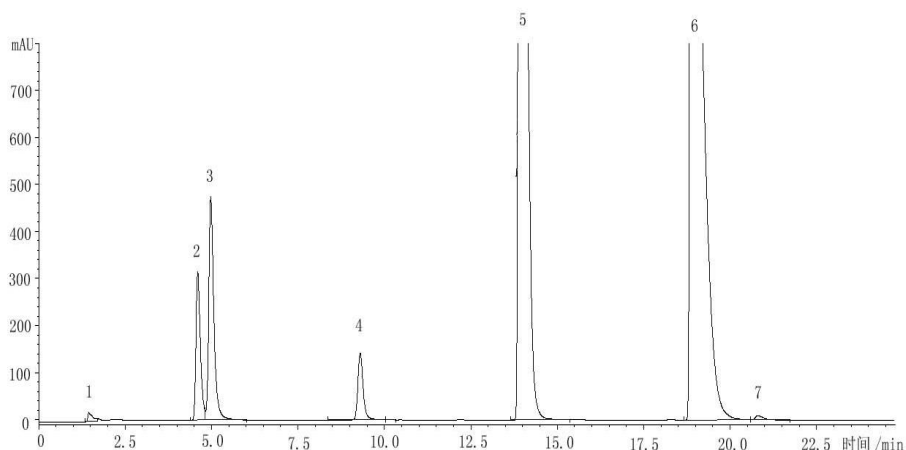
Annex D

(Normative)

Liquid Chromatogram of Amaranth and Retention Time of Components

D.1 Liquid chromatogram of amaranth

See D.1 for liquid chromatogram of amaranth.



1. sodium 6-hydroxy-1,3,6-naphthalenetrisulfonate;
2. sodium 7-hydroxy-1,3-naphthalenedisulfonate;
3. sodium 3-hydroxy-2,7-naphthalenedisulfonate;
4. sodium-1-naphthylamine-4-sulfonate;
5. sodium 6-hydroxy-2-naphthalenesulfonate;
6. amaranth;
7. unknown matter.

Fig. D.1 Liquid chromatogram of amaranth

D.2 Retention time of components of amaranth

See Table D.1 for retention time of components of amaranth.

Table D.1 Retention time of components of amaranth

Peak number	Component name	Retention time (min)
1	Sodium 6-hydroxy-1,3,6-naphthalenetrisulfonate	2.44
2	Sodium 7-hydroxy-1,3-naphthalenedisulfonate	4.59
3	Sodium 3-hydroxy-2,7-naphthalenedisulfonate	4.97
4	Sodium-1-naphthylamine-4-sulfonate	9.29
5	Sodium 6-hydroxy-2-naphthalenesulfonate	13.92
6	Amaranth	18.88

Note: Retention time of components of samples may vary in accordance with apparatuses, separating columns, and even in accordance with injection time, but the elution order of various components is the same.

Annex E

(Informative)

Technical Differences between This Standard and "Food Red No. 2" in Japan's Specifications and Standards for Food Additives (Edition 8)

Table E.1 Technical differences between this Standard and "Food Red No. 2" in Japan's Specifications and Standards for Food Additives (Edition 8)

Chapter in this Standard	Technical differences between this Standard and "Food Red No. 2" in Japan's <i>Specifications and Standards for Food Additives</i> (Edition 8)	Cause
A.4	In Japanese standard, loss on drying (requirement: $\leq 10.0\%$) is separately listed from chloride and sulfate (requirement: $\leq 5.0\%$) and test method is ion chromatography. In this Standard, loss on drying, chloride (based on NaCl) and sulfate (based on Na_2SO_4) are incorporated, requirement is $\leq 15.0\%$ and test method of chloride and sulfate is chemical titration method.	Actual detection proves that ion chromatography used in test of chloride and sulfate brings complex procedure, poor repeatability and incorrect test result, while the classical chemical titration method is simple and correct.
A.6	In Japanese standard, no specific requirement is stipulated for subsidiary colors and test method is spot method, while in this Standard, requirement of subsidiary color is $\leq 3.0\%$, and test method is spectrophotometry after thin-layer chromatography and elution treatment.	Using spectrophotometry to test after thin-layer chromatography and elution treatment can accurately control subsidiary colors and can control inherent quality of products more effectively.
A.9	In Japanese standard, requirement of arsenic (based on As_2O_3) is $\leq 4\text{ mg/kg}$ and test method is limit colorimetry, while in this Standard, requirement for arsenic content is $\leq 1\text{ mg/kg}$ and test	Atomic absorption method can accurately measure content of arsenic.

	method is atomic absorption method.	
A.10	In Japanese standard, requirement of heavy metal (based on Pb) is ≤ 20 mg/kg and test method is limit colorimetry, while in this Standard, content of heavy metal is specified as content of lead, requirement for lead content is ≤ 10 mg/kg and test method is atomic absorption method.	Atomic absorption method can accurately measure content of lead.

END TRANSLATION